Biochemical Pharmacology, 1965, Vol. 14, pp. 1019–1023. Pergamon Press Ltd., Printed in Great Britain.

GLUCURONIDATION OF 5-HYDROXYINDOLE DERIVATIVES *IN VITRO*

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(*Received* 4 *January* 1965; *accepted* 25 *January* 1965)

Abstract—Glucuronidation of 5-hydroxytryptophan-^{14}C (5HTP), 5-hydroxytryptamine-^{14}C creatinine sulphate (5HT), 5-hydroxytryptophol (5HTOH), 5-hydroxyindoleacetic acid (5HIAA) and *p*-nitrophenol was studied in liver, kindey and intestinal homogenates of Sprague-Dawley and Wistar rats.

5-Hydroxyindoles were acceptors of glucuronyl radicals in the following decreasing order: 5HTOH, 5HT, 5HTP, 5HIAA; and *p*-nitrophenol was 10–15 times more active an acceptor than 5HT. Formation of 5HT glucuronide was found also when 5HTP was the substrate and, correspondingly, glucuronides of 5HTOH and/or 5HIAA were formed when 5HT was incubated. Sodium edetate (EDTA) increased the glucuronidation especially in the intestine. EDTA and α-methyldopa decreased the formation of 5HT glucuronide from 5HTP, and EDTA as well as pheniprazine decreased that of 5HTOH and/or 5HIAA glucuronides from 5HT.

Judged from the EDTA experiments the most intense glucuronidation occurred in the intestinal mucous membrane of Sprague-Dawley rats and in the liver of Wistar rats. The glucuronidation of 5-hydroxyindoles ran parallel to that of *p*-nitrophenol in different tissues of both strains as well as in young and adult rats.

IT IS well known that 5-hydroxytryptophan (5HTP), 5-hydroxytryptamine (5HT), 5-hydroxytryptophol (5HTOH) and 5-hydroxyindoleacetic acid (5HIAA) are present, among other 5-hydroxyindole derivatives, as their glucuronides in mammalian urine normally or at least after administration of the corresponding compound and/or its precursor.[1–5] The site of synthesis of these conjugates is not known, although *in vivo* studies in Sprague-Dawley rats indicate that the intestinal wall may play a more important role than the liver in this respect.[5] In the present investigation we observed that glucuronides of 5-hydroxyindole derivatives are formed in a fairly good yield by rat liver, kidney and intestinal homogenates.

EXPERIMENTAL

The animals used were male Sprague-Dawley and Wistar rats aged from six weeks to five months. The tissue homogenates were prepared and glucuronidation of *p*-nitrophenol estimated both in the presence and absence of disodium edetate (EDTA) as previously reported[6] except that the intestinal homogenate was prepared from the isolated mucous membrane and that the concentration of uridine diphosphoglucuronic acid (UDPGA) in the incubation mixture was 1·5 mM instead of 0·5 mM.

5-Hydroxytryptamine-3′-^{14}C creatinine sulphate and 5-hydroxytryptophan-3′-^{14}C (Radiochemical Centre, Amersham) with the specific activities of 176 μc/mM (1 μc/mg

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base) and 440 μc/mM (2 μc/mg), respectively (carriers were products of Fluka AG, Buchs), were used in the quantitative estimation of the glucuronidation of 5HT and 5HTP. 5-Hydroxytryptophol (synthetized as described earlier[5]) and 5HIAA (Fluka AG) were non-radioactive.

The incubation mixture in the determination of the glucuronidation of 5-hydroxyindoles consisted of 100 μl of the same homogenate as used in *p*-nitrophenol experiments, 50 μl of 6 mM UDPGA, 25 μl of 10 mM 5-hydroxyindole compound and 25 μl of 10 M triethanolamine buffer pH 7·5, the final volume being 0·2 ml. The mixture was incubated for 30 min at 37°. The reaction was stopped with 19 volumes of acetone, which precipitated the formed glucosiduronic acid but dissolved most of non-conjugated free 5-hydroxyindoles. After centrifugation, acetone was discarded and the tubes were heated for 30 sec in a 100° water bath to denaturate proteins. The glucuronides were dissolved in water and, after centrifugation, pipetted on two Whatman No. 1 filter papers. The papers were developed with butanol : acetic acid : water (4 : 1 : 5) and isopropanol : ammonia : water (10 : 1 : 1) or butanol : pyridine : water (1 : 1 : 1) and sprayed with *p*-dimethylaminobenzaldehyde reagent. The radioactivity was determined by scanning the chromatograms.[5] The non-radioactive glucuronides of 5-hydroxytryptophol and 5HIAA were only approximately evaluated from the spot size.

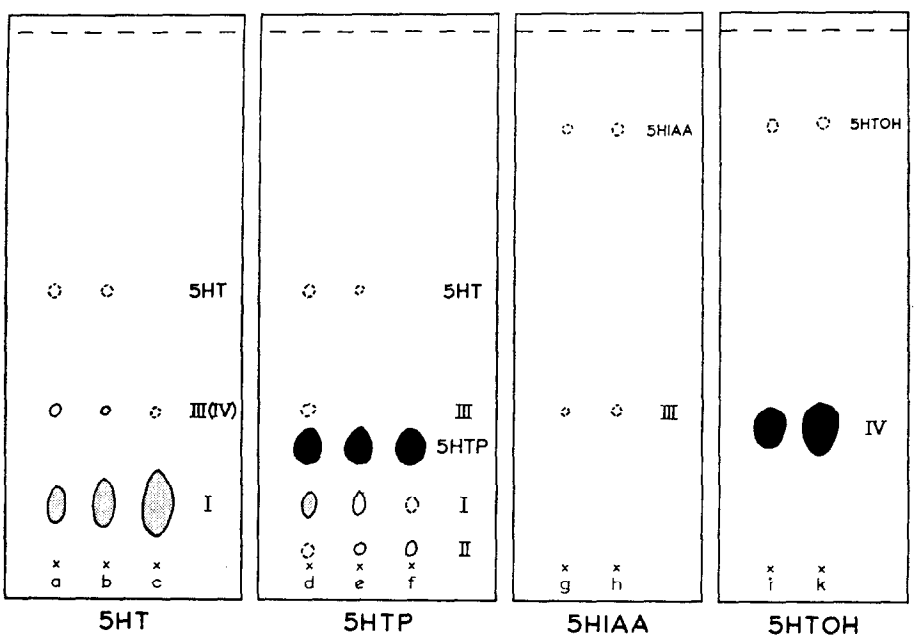

FIG. 1. Scheme of paper chromatograms showing the glucuronidation of 5-hydroxytryptamine (5HT), 5-hydroxytryptophan (5HTP), 5-hydroxyindoleacetic acid (5HIAA) and 5-hydroxytryptophol (5HTOH) in the intestine of a Sprague-Dawley rat. Solvent: butanol-acetic acid-water. (a, d, g and i). = incubations without additions, (b, e, h and k) with disodium edetade, (c) with α-methyldopa, (f) with pheniprazine. (I) 5HT glucuronide, (II) 5HTP glucuronide, (III) 5HIAA glucuronide, (IV) 5HTOH glucuronide. For further details see text.

For identification the spot materials were also hydrolyzed (10 min at 100° in N HCl) and the parent compound shown by paper chromatography. None of the glucuronides were nitrosonaphthol-HNO_2 positive, but they all gave weak colors with nitrosonaphthol-HNO_3,[5] probably because of the hydrolyzing effect of strong nitric acid.

RESULTS

The 5-hydroxyindoles were acceptors of the glucuronyl radical in the following decreasing order: 5HTOH, 5HT, 5HTP, 5HIAA. Figure 1 shows this in the intestine of a Sprague-Dawley rat; similar results in this respect were obtained in the liver and kidney of Sprague-Dawley and also of Wistar rats. The formation of glucuronides of 5HTP and 5HIAA, however, was not constantly found in all the experiments.

In the experiments where 5HTP-3′-^{14}C was used as substrate besides 5HTP glucuronide, an additional radioactive spot with the chromatographic mobility of 5HT glucuronide was also formed. Correspondingly, when 5HT-3′-^{14}C was the substrate a radioactive spot with the chromatographic behavior of 5HTOH and/or 5HIAA glucuronides (these glucuronides did not clearly separate in the solvent system used) was often found in addition of 5HT glucuronide. Both EDTA and α-methyldopa (0·5 mM), a decarboxylase inhibitor, partly inhibited the formation of 5HT glucuronide from 5HTP and increased that of 5HTP glucuronide. Similarly, pheniprazine (PIH; 0·1 mM), a monoamine oxidase inhibitor, as well as EDTA decreased the formation of the conjugates of 5HTOH and/or 5HIAA from 5HT. Table 1 indicates, however, that the formation of "secondary" glucuronides was low (0·1 × 10^{-9} moles) in the intestine compared to that in the liver (1·6 × 10^{-9} moles) and kidney (0·7 × 10^{-9} moles).

TABLE 1. GLUCURONIDATION OF *p*-NITROPHENOL AND 5HT-^{14}C IN THE TISSUE HOMOGENATES OF SPRAGUE-DAWLEY RATS*

Substrate	Liver	Kidney	Intestine
p-Nitrophenol	70·1 ± 8·3	40·3 ± 9·2	66·4 ± 8·9
p-Nitrophenol with EDTA	76·2 ± 2·1	69·5 ± 9·2	108·0 ± 14·4
5HT (total)†	5·7 ± 2·3	4·0 ± 1·5	2·8 ± 1·0
5HT with EDTA (total)	6·0 ± 1·8	4·4 ± 0·6	6·2 ± 1·3
5HT (only 5HT glucuronide)	4·1 ± 1·8	3·3 ± 0·9	2·7 ± 1·0
5HT with EDTA (only 5HT glucuronide)	5·2 ± 1·5	4·3 ± 0·6	6·1 ± 1·3

* Expressed as μmole × 10^{-3}/100 mg of wet tissue/30 min. Values are means ± S.E. of six experiments. (With 5HT in kidney and intestine of five experiments)

† Glucuronides of 5HIAA and 5-hydroxytryptophol formed from 5HT are included.

Table 1 shows that *p*-nitrophenol was conjugated 10–20 times better than 5HT and that EDTA activated glucuronidation particularly in the intestine. Judged from EDTA experiments, intestinal mucous membrane appears to exhibit the highest glucuronidation capacity. Some experiments performed with Wistar rats indicated that glucuronidation of both *p*-nitrophenol and 5-hydroxyindoles was higher in the liver and lower in the kidney and intestine than correspondingly in Sprague-Dawley

rats. Furthermore, few incubations with tissues from young six-weeks-old rats of both strains suggested that not only *p*-nitrophenol but also 5-hydroxyindoles were conjugated better by these animals than by older ones.

DISCUSSION

The most polar 5-hydroxyindoles, 5HIAA and 5HTP, were poor acceptors of glucuronyl radical, while 5HT and particularly 5HTOH, which are more lipophilic at physiological pH, gave a rather good yield of glucuronide. This is in agreement with the theory that glucuronyl transferase is, like other microsomal enzymes, segregated by a lipid barrier which can be penetrated only by substances with some degree of lipid solubility.[7, 8]

In the previous paper[9] we observed that glucuronidation of *p*-nitrophenol was higher in the liver and lower in the kidney and intestine of the Wistar rats than of the Sprague-Dawley rats and that this compound was conjugated better by young than adult animals. These findings are confirmed in the present paper. During the course of this work it could be predicted that the same is true also for 5-hydroxyindoles because glucuronidation of 5HT and *p*-nitrophenol showed a parallel run ($r = 0{\cdot}76$) in spite of a marked quantitative difference. That this prediction is correct, was confirmed finally by some experiments done with adult Wistar rats and young animals of both strains. This apparent association observed between the glucuronidation of *p*-nitrophenol and 5-hydroxyindoles suggests that the same enzyme may conjugate all these compounds. Whether a different solubility of *p*-nitrophenol and 5HT to the microsomal lipid barrier is the explanation for the 10–20-fold difference in their glucuronidation remains unknown.

The increase of glucuronidation of 5-hydroxyindoles by EDTA may partly be due to a decrease of their further metabolism. It is well known that EDTA in high concentrations inhibits 5HTP decarboxylase[10] and perhaps also degradation of 5HT. On the other hand, EDTA had little effect on the formation of secondary glucuronides in the intestine whereas total glucuronidation of 5HT was increased 2-fold or more. This means that some additional mechanism is operating. Although the release of inorganic phosphate from UDPGA was not measured in the present study, we know from earlier work that EDTA inhibits UDPGA-pyrophosphatase, thus leading through more complete substrate saturation to a better glucuronidation.[6, 11] UDPGA-pyrophosphatase activity is known to be particularly high in the intestine. Neither is the possibility that newly formed glucuronides are partially hydrolyzed by β-glucuronidase excluded. Some evidence is obtained that EDTA, under the conditions used in the present study, may inhibit this enzyme.[6]

The monoamine oxidase inhibitors are found to inhibit the drug-metabolizing enzymes of rat microsomes,[7, 12] however, in this work the small PIH dose used did not seem to inhibit the glucuronidation.

Because 5HIAA was a rather poor acceptor of glucuronyl radical and 5HT glucuronide is a substrate of monoamine oxidase,[2, 5] a part of the 5HIAA glucuronide found in urine after 5HT administration may be formed from the glucoronide of 5HT or possibly from that of 5HTOH. On the other hand, the glucuronide of 5HTOH is probably a conjugation product of 5HTOH itself also after 5HT administration. Nothing is known of the possible glucuronide of the corresponding aldehyde. The

following outline of the glucoronide formation of 5-hydroxyindoles seems to be valid (intense glucuronidation indicated with a thick arrow and unknown route with dotted arrow):

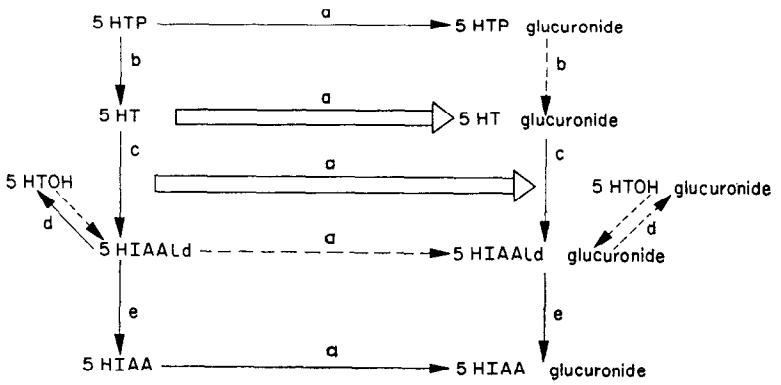

FIG. 2. a = glucuronyl transferase; b = decarboxylase; c = momoamine oxidase; d = alcohol dehydrogenase; e = aldehyde dehydrogenase; 5HIAAld = 5-hydroxyindole acetaldehyde

Acknowledgements—We are indebted to Merck, Sharp & Dohme, Ltd. for α-methyldopa and to A. B. Leo for phenelzine.

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